

BIRCH, STEWART, KOLASCH & BIRCH, LLP

INTELLECTUAL PROPERTY LAW
8110 GATEHOUSE ROAD
SUITE 500 EAST
FALLS CHURCH, VA 22042-1210
U S A
(703) 205-8000

FAX: (703) 205-8050
(703) 698-8590 (G IV)

e-mail: mailroom@bskb.com
web: http://www.bskb.com

CALIFORNIA OFFICE:
COSTA MESA, CALIFORNIA

THOMAS S. AUCHTERLONE
JAMES T. ELLER, JR.
SCOTT L. LOWE
MARK J. NUEL, Ph.D.
D. RICHARD ANDERSON
PAUL C. LEWIS
MARK W. MILSTEAD*
RICHARD J. GALLAGHER
JAYNE M. SAYDAH*

REG. PATENT AGENTS
FREDERICK R. HANDREN
MARYANNE ARMSTRONG, Ph.D.
MAKI HATSUMI
MIKE S. RYU
CRAIG A. McROBBIE
GARTH M. DAHLEN, Ph.D.
LAURA C. LUTZ
ROBERT E. GOOZNER, Ph.D.
HYUNG N. SOHN
MATTHEW J. LATTIG
ALAN PEDERSEN-GILES
C. KEITH MONTGOMERY
TIMOTHY R. WYCKOFF
KRISTIL L. RUPERT, Ph.D.
LARRY J. HUME
ALBERT LEE
HRAYR A. SAYADIAN, Ph.D.

TERRELL C. BIRCH
RAYMOND C. STEWART
JOSEPH A. KOLASCH
JAMES M. SLATTERY
BERNARD L. SWEENEY*
MICHAEL K. MUTTER
CHARLES GORENSTEIN
GERALD M. MURPHY, JR.
LEONARD R. SVENSSON
TERRY L. CLARK
ANDREW D. MEIKLE
MARC S. WEINER
JOE MCKINNEY MUNCY
ROBERT J. KENNEY
DONALD J. DALEY
JOHN W. BAILEY
JOHN A. CASTELLANO, III
GARY D. YACURA

OF COUNSEL:
HERBERT M. BIRCH (1905-1996)
ELLIOT A. GOLDBERG*
WILLIAM L. GATES*
EDWARD H. VALANCE
RUPERT J. BRADY (RET.)*
F. PRINCE BUTLER
FRED S. WHISENHUNT

*ADMITTED TO A BAR OTHER THAN VA

Date: October 2, 2000

Docket No.: 2761-0139P

Assistant Commissioner for Patents
Box PATENT APPLICATION
Washington, D.C. 20231

Sir:

As authorized by the inventor(s), transmitted herewith for filing
is a patent application applied for on behalf of the inventor(s)
according to the provisions of 37 CFR 1.41(c).

Inventor(s): BHADRA, Ranjan
PAL, Bikash Chandra; DAS, Krishna
BHATTACHARAYA, Samir

For: A HERBAL COMPOSITION FOR TREATING ASTHMA

Enclosed are:

- X A specification consisting of 22 pages
- _____ sheet(s) of _____ drawings
- _____ Certified copy of Priority Document(s)
- X Executed Declaration in accordance with 37 CFR 1.64 will follow
- _____ A verified statement to establish small entity status under 37
CFR 1.9 and 37 CFR 1.27
- _____ Preliminary Amendment
- X Information Sheet
- _____ Information Disclosure Statement, PTO-1449 with reference(s)

Other _____

The filing fee has been calculated as shown below:

			LARGE ENTITY		SMALL ENTITY
FOR	NO. FILED	NO. EXTRA	RATE FEE		RATE FEE
BASIC FEE	***** ***** *****	***** ***** *****	***** ***** \$710.00 *****	or	***** ***** \$355.00 *****
TOTAL CLAIMS	40 - 20 =	20	x18 =\$ 360.00	or	x 9 = \$ 0.00
INDEPENDENT	5 - 3 =	2	x80 =\$ 160 .00	or	x 40 = \$ 0.00
MULTIPLE DEPENDENT CLAIM PRESENTED <u>no</u>			+270 = \$ 0.00	or	+135 = \$ 0.00
			TOTAL \$1230.00		TOTAL \$ 0.00

- X The application transmitted herewith is filed in accordance with 37 CFR 1.41(c). The undersigned has been authorized by the inventor(s) to file the present application. The original duly executed patent application together with the surcharge will be forwarded in due course.
- X A check in the amount of \$1,230.00 to cover the filing fee and recording fee (if applicable) is enclosed.
- _____ The Government Filing Fee will be paid at the time of completion of the filing requirement.
- _____ Please charge Deposit Account No. 02-2448 in the amount of \$_____. A triplicate copy of this transmittal form is enclosed.
- X Send Correspondence to: BIRCH, STEWART, KOLASCH & BIRCH, LLP
P. O. Box 747
Falls Church, Virginia 22040-0747

(703) 205-8000
GMM/amr

Respectfully submitted,

By

~~GERALD M. MURPHY, JR.~~

Reg. No. 28,977

P. O. Box 747

Falls Church, Virginia 22040-0747

Field of the invention

The present invention relates to the identification of a novel use of the principles extracted from the plant *Murraya koenigii* for treatment of asthma. This invention provides a process for the extraction of the principles from the plant *Murraya koenigii*. The invention also provides novel compositions useful in the treatment of asthma and methods for the treatment of asthmatic conditions using the said composition.

Background and prior art references

Respiratory diseases such as asthma are reaching epidemic proportions in both the developed and developing world (Nature, Vol. 402, Supplement, and No. 6760, 1999. A special supplement on allergy and asthma). In countries such as Britain and Australia, the respiratory problem translates to 1 in 4 children under age of 14 years having asthma. The disease causes distress and misery in millions often at a time in their lives when they should be most active. Asthma interferes with sleep, intellectual functioning and recreational activities. At one extreme, asthma can be life threatening leading to occurrence to deaths that are avoidable.

Expert panel report has offered guidelines for diagnosis and management of asthma (JAMA Asthma Information Center, www.amaasssm.org/special/asthma/treatmnt/guide/guideline/guidelin.htm). Control of factors contributing to asthma severity is another avenue for management (JAMA Asthma Information Center, www.amaasssm.org/special/asthma/treatmnt/guide/guidelin/comp2/comp2.htm). several therapies and medications have also been suggested (JAMA Asthma Information Center, www.amaasssm.org/special/asthma/treatmnt/guide/guidelin/comp3/medicat/meditoc.htm and www.amaasssm.org/special/asthma/treatmnt/guide/guidelin/comp3/comp3.htm). Immunotherapy is another route for the treatment (JAMA Asthma Information Center, www.amaasssm.org/special/asthma/treatmnt/guide/guidelin/comp2/immunoth.htm). Complementary alternative medicine is also being tried (JAMA Asthma Information Center, www.amaasssm.org/special/asthma/treatmnt/guide/guidelin/comp3/medicat/alternat.hmt).

However, no satisfactory medication for cure of the disease has been found.

Herein the active factors in *Murraya koenigii* was prepared and used under in vitro system to show its usefulness for relief, treatment and cure of asthmatic problem. The plant *Murraya koenigii* has been studied and reported to have various medicinal values (Chakraborty,

M.P Phytochemistry 1997, Oct; Khan B. A, Indian J of Expt. Biol. 1997 Feb.; Khan B A Indian J of Physiology and Pharmacology 1996, Apr.; Khan BA Plant Foods Hum. Nutr. 1996 Jun).

The leaf of this plant is widely used in various food preparations in India. Further, the plant is ubiquitous. Thus the prior art suggests that the extracts of the plant have several medicinal and other properties which have been widely investigated. This is adequate proof that the extracts of the plant can be safely consumed by humans without any side effects.

The applicants undertook a study on the extracts of this plant to identify the therapeutic principles of the plant. During their study, the applicants discovered the presence of the active principles in the leaf of *Murraya koenigii* which surprisingly were found to be useful in the treatment and cure of asthma.

Objectives of the invention

The main objective of the present invention is to provide a method for the treatment of asthmatic conditions employing active principles extracted from the plant *Murraya koenigii*.

Another object is to provide a process for the isolation of active principles from the plant *Murraya koenigii* and use the same for relief, treatment and cure of asthma.

Yet another object is to provide a simple, fast and inexpensive process to obtain a mixture of active (four) compounds possessing biological activities useful in the treatment of asthma.

Still another object is to provide novel compositions containing active principles extracted from the plant *Murraya koenigii* and useful in the treatment of asthma.

Summary of the invention

Accordingly, the present invention provides a method for the treatment of asthma, employing principles extracted from the plant *Murraya koenigii*. The invention also provides novel compositions useful in the treatment of asthma and methods for the extraction of active principles from the plant *Murraya koenigii*.

Detailed description of the invention

Accordingly, the invention provides a process for preparing an extract from the plant *Murraya koenigii*, useful in the treatment of asthma, said process comprising the steps of pulverising plant materials obtained from plant *Murraya koenigii*, extracting the plant material with a solvent at

ambient temperature, concentrating the extract by filtering and evaporating it under reduced pressure and lyophilizing the concentrate to obtain a lyophilized extract containing active principles of the plant *Murraya koenigii*.

In an embodiment, the plant materials are obtained from plant parts of *Murraya koenigii* selected from garden fresh leaves or leaves dried under shade.

In another embodiment, the leaves are pulverized by conventional methods to get homogenized leaves.

In yet another embodiment, the plant materials are extracted with solvents selected from hydrocarbon solvents, chlorinated solvents, ester solvents, ketonic solvents, alcohols, water and buffers.

In still another embodiment, the solvents are selected from the group consisting of petroleum ether (BP 40 – 60°C), petroleum ether (60°C - 80°C), benzene, pentane, hexane, chloroform, dichloromethane, carbon tetrachloride, diethyl ether, tetrahydrofuran, dioxane, acetone, cyclopentanone, ethyl acetate, ethyl formate, methanol, ethanol, n-butanol, water and buffers.

In another embodiment, the concentration of the extract is effected by filtering and evaporating the solvents under reduced pressure at a temperature range of 20°C – 80°C preferably at ambient temperature and lyophilizing the concentrate by conventional methods to obtain mixtures of the active factors.

In yet another embodiment, the extract obtained from the plant *Murraya koenigii* comprises active principles which dark colored solids soluble in dimethylsulfoxide.

In another embodiment, the active principles obtained from the plant *Murraya koenigii* are biocompatible and non-toxic in nature. The active principles have R_f values 0.73, 0.60, 0.34 and 0.14 in chloroform and methanol in the ratio 19:1 and R_f values 0.60, 0.38, 0.24 and 0.15 in the chloroform. The active principles have four peak with retention time 3.37, 3.49, 4.0 and 5.69 in methanol as solvent at 254nm. The extraction process is carried out for a period ranging from 1 – 120 hrs, preferably between 12 – 16 hrs.

Further, the invention also provides pharmaceutical composition useful in the treatment of asthma, said composition comprising an effective amount of extract obtained from the plant *Murraya koenigii* together with, or optionally associated with a pharmaceutically acceptable

additive. The additives used in the composition comprise powder or extracts of plants selected from *M. paniculate* Linn, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A.vasica* Nees, *E hirta*, and *M. koinegii*. These additives are present in the range of 80-100 mg of *M. paniculate* Linn, 40-60 mg of *H. abelmoschus*, 38-62 mg of *T. ammi*, 7-13 mg of *S. aromaticum*, 85 - 115 mg of *A.vasica* Nees and 90-110 mg of *E hirta*.

In an embodiment, the composition comprises

<i>M. paniculata</i> Linn. Syn. <i>M exotica</i> (KAMINI)	90mg
<i>H. abelmoschus</i> (JOWAN)	50mg
<i>T. ammi</i> (LAVANGA)	50mg
<i>S. aromaticum</i> (BASAK)	10mg
<i>A.vasica</i> Nees (PUSITOA)	100mg
<i>E.hirta</i>	100mg
<i>M. koinegii</i> (Suravi Neem)	100mg

In the said composition, the extract of the plant *M. koinegii* is present in the range of 87-105 mg per dose. In the said composition, the extract of the plant *M. koinegii* comprises active principles which are dark colored solids, soluble in dimethylsulfoxide. The active principles have R_f values 0.73, 0.60, 0.34 and 0.14 in chloroform and methanol in the ratio 19:1 and R_f values 0.60, 0.38, 0.24 and 0.15 in the chloroform. These principles have four peaks with retention time 3.37, 3.49, 4.0 and 5.69 in methanol as solvent at 254nm. The said active principles obtained from the plant *M. koinegii* exhibit antioxidant property i.e. O_2 inhibition.

Further, the invention provides a method for the treatment of asthma, said method comprising the steps of administering an effective amount of the composition as claimed in claim 13 to a subject in need thereof.

In an embodiment, the lyophilized extract obtained from *Murraya Koenigii* is administered alone or alongwith other conventional additives for the treatment of asthma. In still another embodiment, the mode of administration is oral for the treatment of mild or acute asthma.

In yet another embodiment, the dosage level of the composition, comprising the extract from the plant *Murraya Koenigii* is between 325-600 mg twice daily for the period ranging from 3 to 30 days.

In another embodiment, the dosage level is in between 325-600 mg twice daily for the period ranging from 3 to 15 days for mild asthmatic condition, and 15 – 30 days for acute asthmatic condition.

In still another embodiment, the additives are selected from *M. paniculate Linn*, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A.vasica Nees*, *E hirta*, and *M. koinegii*.

As said above, the additives are present in a range of 80-100 mg of *M. paniculate Linn*, 40-60 mg of *H. abelmoschus*, 38-62 mg of *T. ammi*, 7-13 mg of *S. aromaticum*, 85-115 mg of *A.vasica Nees*, 90-110 mg of *E hirta*, and 87-105 mg of *M. koinegii*. per dose.

In another embodiment, the additives obatined from *M. paniculate Linn*, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A.vasica Nees*, *E hirta*, and *M. koinegii* are administered to include properties such as antidiahorial, antiseptic, carminative, stimulation, anti-cough, anti- bronchitis and nourishment.

In another embodiment, the additives are obtained from :

M. paniculate Linn (bark or root), *H. abelmoschus* from dried flower buds, *T. ammi* from leaves, *S. aromaticum* from whole plant *A.vasica Nees* from root, *E hirta* from bark, and *M. koinegii* from leaves.

In addition, the invention provides an anti-oxidant composition for human beings and animals, said composition comprising a effective amount of extract obtained from the plant *Murraya Koenigii* together with or optionally, associated with pharmaceutically acceptable additives.

In still another embodiment, the additives comprise powder or extracts of plants selected from *M. paniculate* Linn, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A.vasica* Nees, *E hirta*, and *M. koinegii*.

In an embodiment, the additives are present in a range of 80-100 mg of *M. paniculate* Linn, 40-60 mg of *H. abelmoschus*, 38-62 mg of *T.ammi*, 7-13 mg of *S. aromaticum*, 85-115 mg of *A.vasica* Nees, 90-110 mg of *E hirta*, and 87-105 mg of *M. koinegii*. per dose of the anti-oxidant composition.

As said above, the active factor(s) in *Murraya koenigii* useful for relief, treatment and cure of asthmatic problem, the preparation of which comprises drying powdering, and extracting the dried leaves of the plant, *Murraya koenigii*, in a percolator at an ambient temperature using appropriate solvents and concentrating the extract under reduced pressure and finally lyophilizing the concentrate to make the active factors.

In the present invention, active factor(s) in *Murraya koenigii* for the treatment and cure of asthmatic problem are prepared from the leaves of the plant by drying, powdering and extracting, in a percolator at an ambient temperature using appropriate solvents and concentrating the extract under reduced pressure and finally lyophilizing the concentrate to make the active factors.

In an embodiment of the present invention, the active factors from the plant material to be used as an oral preparation for relief, treatment and cure of asthmatic problem.

In another embodiment of the present invention, the leaf of *Murraya koenigii* used was garden fresh or fresh leaf dried under sun or shade.

In still another embodiment of the present invention, plant material was used for the extraction with appropriate solvents such as methanol or water or buffers in a percolator.

In yet another embodiment of the present invention, the plant extract made by the methanol/appropriate solvent or water was concentrated under reduced pressure to get the mixture of active factor.

In yet another embodiment of the present invention, the concentrate of the plant extract was lyophilized to make the factors free of solvent or water

In yet another embodiment of the present invention, lyophilized solid obtained as the mixture of active factors present in the leaf of *Murraya koenigii* was proposed to be used for relief, treatment and cure of asthmatic problem.

The method of preparation of the active factors comprises the following

- 1) collecting the fresh leaves from the local suppliers,
- 2) drying the leaves under shade to a moderate degree or to take the fresh leave as the starting material,
- 3) powdering the dried or homogenizing the fresh leaves in the apparatus known in the art,
- 4) putting the powder or homogenate in a percolator under the bulk of appropriate solvents; choosing hydrocarbon solves such as petroleum ether (B.P 40-60⁰C), petroleum ether (B.P 60-80⁰C), pentane, hexane, benzene etc.; chlorinated solvents like chloroform, dichloromethane, carbon tetrachloride etc.; etherial solvents such as diethyl ether, tetrahydrofuran, dioxane etc.; ketonic solvents such as acetone, cyclopentanone etc.; ester solvents such as ethyl acetate, ethyl formate etc.; all alcohol such as methanol, ethanol, n-butanol etc.; water and buffers,
- 5) extracting the percolated plant material using a percolator or the apparatus or equipment currently known in the art over a period of time ranging from 1 to 120 hours,
- 6) evaporating the solvent under reduced pressure using an apparatus or equipment currently known in the art at a temperature ranging from 20 to 80°C,
- 7) lyophilizing or drying the material in the apparatus or equipment currently known in the art,
- 8) storing the processed material in a cool and dry place in an air tight container, and
- 9) evaluating the bioactivity of the material.

The following examples are given by way of illustration and therefore should not be construed to limit the scope of the present invention.

Collection of Plant material

The leaves of *M. koenigii* were collected from shrubs in the month of March-August 2000 from different areas of West Bengal, India. A voucher specimen is deposited at the department of Medicinal Chemistry at the Indian Institute of Chemical Biology, 4, Raja S. C. Mallick Road, Calcutta- 700 032.

Preparation of the active factors or material

Example – 1

Fresh leave containing branches of *Murraya koenigii* collected, cleaned and washed with water after getting from the local supplier and used as starting material. It was air-dried under shade and drying continued till it was brittle to make suitable for powdering in grinder-mixture.

87 gm. of the powdered leaves of *Murraya koenigii* was placed in a glass percolator (5-litr. capacity) with the addition of 1000 ml. of methanol so as to submerge the material satisfactorily. This was kept for 12 to 16 hrs (over night) at room temperature. Filtering the extract through Whatman No.1 filter paper collected the percolate. The process of extraction was repeated four times and the combined extract was evaporated to dryness under reduced pressure in a rotary evaporator keeping the temperature at 40°C (bath). The solid residual matter left was viscous in appearance and it was further dried by lyophilization. The yield was 14.84 gm. and the material was tested and found biologically active in both neutrophil or ex vivo blood system.

Example – 2

The leaves of *Murray koenigii* air dried and weighing 90 gms , was powdered and taken in a percolator. It was dipped in 800-ml chloroform. The submerged material was allowed to be extracted by the solvent for 12 to 16 hours (over night) The extract was filtered through Whatman No.1 filter paper and collected; this process was repeated for three times. The extract was evaporated to dryness in flash evaporator under reduced pressure at 40°C. The residual substance was then dried in a desiccator under high vacuum and the solid mass weighing 11.5gm was tested and found biologically active.

Example – 3

150 gm of fresh leaves of *Murraya koenigii* thoroughly washed in sterile water was homogenized with 750 ml of glass distilled water in a mixture-blender and then sonicated in an ultra sonic bath with 5 burst, each for 15min. Filtering through Whatman no.1 filter paper separated the material extracted in water. This type of treatment for extraction was repeated for three times. The combined extract was lyophilized yielding a powdered material, 11gm in weight. This was then tested for biological activity.

Properties of the materials

The biologically active material obtained by examples 1 or 2 or 3 has the following properties:-

1. The dried solid prepared as stated above was a dark coloured material soluble in DMSO or dimethyl sulfoxide.
2. Thin layer chromatography of the active material shows four spots having Rf.0.60, 0.38, 0.24, and 0.15 in the solvent system of chloroform and Rf 0.73,0.60,0.34 and 0.14 in solvent system chloroform and methanol (19:1).
3. The HPLC analysis of the active material using Intersil ODS-3 (4.6x250mm) analytical column, solvent system methanol and a flow rate 1.0ml /min., detection at 254nm resolved the material into four peaks with the retention time 3.37, 3.49, 4.00, and 5.69 mins.

EVALUATION OF THE ACTIVITY IN AN IN VITRO SYSTEM

Biological evaluation was carried out as per the following procedure.

a) *Inhibition of arachidonic acid oxidation in presence of the active material by neutrophil*

Human peripheral blood neutrophil was prepared by standard method [Downey, G.P., Fukushima, T., Fialkow, L. and Waddel, T.K. (1995) Semin. Cell Biol. 6 345]. In brief, heparinised blood treated with 2% gelatin (in normal saline) for half an hour to allow the RBC separation at the bottom. The rest of the mixture was taken and subjected to gradient separation using Histopaque and carrying out the centrifugation (at 1400 rpm at room temp. for 12 min.). The neutrophil available as a separate layer at the bottom was collected and then made into

suspension in sterile PBS or phosphate buffered saline.

(i) The neutrophil (1×10^4 /well of 24 well tissue culture plate) was treated with active material (20 (g/ml) for 60 min., while control did not contain any active material.

(ii) The neutrophil primed with Phorbol Myristic Acetate (PMA) (10(M) for 30 min. Followed by addition of active material (20 (g/ml) or without it for 30 mins.

(iii) The neutrophil treated with active material (20 (g/ml) for 30 min. followed by PMA (10(M) activation for 30 min.

The oxygen consumption by the treated neutrophil was measured after ten minutes of the addition of arachidonic acid 10(1 (12.2 mg/ml in absolute alcohol) (Method. Enzymol. Ed. By Murphy, R.C, and Fitzpatrick F.A. vol 187 pp-268), in 2 ml volume of neutrophil suspension. The assay of the oxygen consumption was carried out by oxygen sensor (of Hansatec with O₂ electrode control having Clark type probe). The results of inhibition of oxygen consumption due to arachidonic acid oxidation are given in Table-1

Table-I

Inhibition of arachidonic acid oxidation by neutrophil

Treatment	Micromole oxygen consumed/10 min/	% of inhibition of O ₂ consumption by active material with respect to	
		stimulation	without stimulation
A .PBS 60 min.	16.08	NA	
B Active extract 60 min in '1'	12.85	----	---
C. PMA 30 min.	39.58	----	20
D. Calcium ionophore 30 min.	64.01	-----	

E. Active extract 30min + PMA 30 min.	21.42	46	
F. Active extract 30min.+Calcium ionosphere 30 min	25.3	60	

Calcium ionophore gave better results as there was about 60% inhibition while PMA effects 46% inhibition of O₂ consumption by active material present in *Murraya koenigii* leaf under in vitro neutrophil test. In both the stimulation remarkable inhibition of O₂ consumption indicates the efficacy of the material.

The results in Table-1 illustrated that the active factors in *Murraya koenigii* leaf acted as an inhibitor of oxygen consumption in presence of normal as well as activated human neutrophil and arachidonic acid. It suggested that oxidation of arachidonic acid is strongly inhibited by active factors present in *Murraya koenigii* leaves. The leukotrienes are the biological mediators of asthma and the oxidation of arachidonic acid is the rate-determining step for the synthesis of the leukotrienes. The strategy of asthma drug development is to search or synthesize compounds or prepare substances that inhibit the synthesis of leukotrienes or precisely cause the inhibition of the oxidation of arachidonic acid. The active material or compounds prepared here not only inhibit the oxidation of arachidonic acid but also expected to be free of toxicity and compatible for use in human as the plant leaves are extensively used as food ingredients. Hence it qualifies as a drug for asthma.

b) Inhibition of arachidonic acid oxidation in presence of the active material by Human ex vivo Blood.

500µl of heparinised human whole blood was taken in each well of a 6-well micrometer plate to which the *Murraya koenigii* active material or none was added to have a final concentration of 50(g/ml when the total volume was adjusted to 2 ml. with PBS. The plates were incubated for 2 hours with constant shaking. Next 10µl of arachidonic acid (12.2gm/ml in absolute alcohol stored under argon at -200C; Method. Enzymol. Ed By Murphy, R. C, and Fitzpatrick F.A. vol. 187 pp-268) was added to each well for 10 mins. Prior to the addition of

Phorbol Myristic Acetate (PMA, 10(M) or calcium ionophore A23187 (20(g/ml), (Spaethe S.M, Snyder D.W, Pechous P.A, Clarke T, VanAlstyne E.L Biochem. Pharmacy. 43; 1992,377-82). After 30 mins of the addition of the stimulator, a YSI Clark oxygen probe containing oxygen-monitoring equipment monitored oxygen consumption.

Table –2

Inhibition of arachidonic acid in whole human ex vivo blood in presence of *Murraya koenigii* leaf material

Addition	Oxygen consumption (in nmol) /10 mins	Inhibition % of control
A. None N1(PMA treated)	39.99	-----
B. None N2 (Ca+ionophore)	62.41	-----
C. None N3 (patient's blood)	32.15	-----
D. Whole leaf in N1 blood	22.1	45
E. Water extract in N1 blood	18.56	54
F. Methanol extract in N1 blood	10.70	73
G. Methanol extract in N2 blood	18.21	73
H. Methanol extract in N3 blood	11.70	70
Chloroform extract in N1 blood	11.11	72

* Patient having Eosinophil count 13 with 7 years of chronic asthma.

The Nitro Blue Tetrazolium reduction test was also found highly effective in presence of the leaf preparation demanding it to be a powerful antioxidant.

Again, the Nitro Blue Tetrazolium (NBT) reduction test was carried out as per the standard procedure [Rouiller,Y.B., and Mauel J.(1987) Infec.Immun. 55, 587]. Neutrophil was prepared and treated with or without PMA (30 min.) and followed by leaf extract (60 min.) or

without it. Then 100(1 of NBT solution (3mg /ml) was added and cells were incubated for various intervals (0 to 120 mins). Formazan deposition was measured at 550 nm in an ELISA reader (Labsystem Multi Skan MS). The results showed that the activation of neutrophil with PMA was strongly inhibited about 6 times in the presence of the active material from the Murray koenigii leaves at 120 min. The leaf preparation has strong antioxidant property which lend a firm support to recuperate from the condition occurs during asthma.

More over we shall see that the freshness is a must as because one of volunteer patients takes cooked the leaves in his food but his severe attack was not influenced with such a material.

LOCAL NAMES	PARTS SELECTED	AMOUNTS
KAMINI (<i>M. paniculata</i> Linn. Syn. <i>M. exotica</i>)	Bark of root	80 - 100
LATAKASTURI (<i>H. abelmoschus</i>)	Seed	40 - 60
JOWAN (<i>T. ammi</i>)	Fruit	38 - 62
LAVANGA (<i>S. aromaticum</i>)	Dried flower buds	7 - 13
BASAK (<i>A. vasica</i> Nees)	Leaves	85 - 115
PUSITOA (<i>E. hirta</i>)	Whole plant	90 - 110
Suravi Neem <i>M. koinegii</i>	Root bark and leaves	87 - 105

The leaves of these plants are frequently used as flavor enhancer in South Indian cooked food. However, heating of the leaves denatures its anti-asthmatic property. Its use as a cooking item or in food recipe is known for more than centuries and no untoward occurrence is yet evident indicating all components of this leaf as totally safe for human consumption. Use of this

plant especially in South Indian food never produced bowel discomfort or vomiting or stomach upset or any other problem. From all these information it is quite convincing that any preparation from this leaf as a drug for asthma will be absolutely safe.

The leaves of the plants are used as flavor enhancer in cooked food in most of the parts of India. The use is known for centuries and till date no untowards effects is reported from any corner. Especially it is neither bitter in taste nor odd in taste. After taking the leaf preparation no bowel discomfort or vomiting or stomach upset or any gastrointestinal problem was reported from any of the healthy volunteers. It was taken twice daily over a period of 30 days and found totally satisfactory without any complain from any of the healthy volunteers.

Five patients volunteer (both female and male) were chosen with respect to their asthmatic condition depending on their sensitivity to the different causative agents that manifest their asthma.

Case-I

A female patient known for dust and cold sensitive severe asthma suffering for last seven years. Chest tightness, wheeze, cough, severe shortness of breathing, night sleeplessness and huge mucus secretion during late night hours were the primary symptoms. She had to take inhaler twice or thrice daily.

For medication two spoonful whole plant preparation were taken by her twice daily for fifteen days that observations were as follows:

- a) Currently she did not have to take inhaler except occasionally once in a day.
- b) Wheeze and cough had been totally stopped.
- c) She is now completely free from chest tightness.
- d) She appears physically normal and capable of taking strenuous walking like stepping up staircase to cross floors upto third level.

Case – II

Two volunteer patient (one male and another female) with genetic predisposition for asthma had sleepiness during night time and very prone to asthmatic condition for the sensitive

to the city pollution. Shortness of breathing was their main complains and one has to take inhaler when there was severe night attack. They followed the medication like the case –I for one month.

- i) Upto nine month of observation they did not have nay asthmatic attach,
- ii) No shortness of breathing was reported by them,
- iii) In case of male patient his smoking was a risk enhancer of the frequent episode of asthmatic attack. But he did not have any breathing discomfort for smoking even exceeding the regular schedule.
- iv) Female patient had shortness of breathing when she took long walk or climbing of the stair cases only upto a single floor but she did not have any trouble of breathing even when she climbed upto third level.

Case - III

Another young volunteer patient who has periodically severe attack with acute shortness of breathing and it was usual practice that he had to take in hospital for fresh oxygen supply to ease out breathing acuteness.

During one such episode of acute attack of shortness of breathing, he took the leaf preparation and the inference are:

- i) He did not have to attend hospital,
- ii) Since then he took this medication for a month, and
- iii) His every day activity has been increased remarkable till date there is no occurrence of highly acute respiratory distress.

Case-IV

An aged woman patient suffering from sleeplessness during night and extreme shortness of breathing and showing no response to the modern medication including inhaler.

She was medicated with the leave preparation taking twice daily for fifteen day and her condition has improved remarkably.

Thus, the application herein providing a sample, fast inexpensive process for preparation of a plant material from an abundantly available plant for relief, treatment and cure of asthmatic problem satisfactorily. The method of administration of the plant material is also simple. The success rate is 100% among the volunteer. There has been no case of relapse during the observation time of 6 months. No adverse or side effect was found after the treatment.

The major advantages of the present invention are:

1. It is expected to provide relief, treatment and cure of asthmatic problem satisfactorily.
2. It is prepared from a plant material, which is used in food for centuries.
3. The plant material is biocompatible and non-toxic as it is consumed along with food.
4. The plant is abundantly available.
5. Method of preparation of the active material /principle is simple, fast and inexpensive.
6. Its cultivation and propagation will be easy and it grows in all kinds of soil.

CLAIMS:

1. A process for preparing of an extract from the plant *Murraya koenigii*, useful in the treatment of asthma, said process comprising the steps of pulverising plant materials obtained from plant *Murraya koenigii*, extracting the plant material with a solvent at ambient temperature, concentrating the extract by filtering and evaporating it under reduced pressure and lyophilizing the concentrate to obtain a lyophilized extract containing active principles of the plant *Murraya koenigii*.
2. A process as claimed in claim 1 wherein the plant materials are obtained from plant parts of *Murraya koenigii* selected from garden fresh leaves or leaves dried under shade.
3. A process as claimed in claim 1 wherein the leaves are pulverized by conventional methods to get homogenized leaves.
4. A process as claimed in claim 1 wherein the plant materials are extracted with solvents selected from hydrocarbon solvents, chlorinated solvents, ester solvents, ketonic solvents, alcohols, water and buffers.
5. A process as claimed in claim 4 wherein the solvents are selected from the group consisting of petroleum ether (BP 40 – 60°C), petroleum ether (60°C - 80°C), benzene, pentane, hexane, chloroform, dichloromethane, carbon tetrachloride, diethyl ether, tetrahydrofuran, dioxane, acetone, cyclopentanone, ethyl acetate, ethyl formate, methanol, ethanol, n-butanol, water and buffers.
6. A process as claimed in claim 1 wherein the concentration of the extract is effected by filtering and evaporating the solvents under reduced pressure at a temperature range of 20°C – 80°C preferably at ambient temperature and lyophilizing the concentrate by conventional methods to obtain mixtures of the active factors.
7. A process as claimed in claim 1, where in the extract obtained from the plant *Murraya koenigii* comprises active principles which dark colored solids soluble in dimethylsulfoxide.
8. A process as claimed in claim 1, wherein the active principles obtained from the plant *Murraya koenigii* are biocompatible and non-toxic in nature.
9. A process as claimed in claim 1, wherein the pharmaceutical composition is used for inhibition of arachidonic acid oxidation.

10. A process as claimed in claim 1, wherein the active principles have R_f values 0.73, 0.60, 0.34 and 0.14 in chloroform and methanol in the ratio 19:1 and R_f values 0.60, 0.38, 0.24 and 0.15 in the chloroform.
11. A process as claimed in claim 1 wherein the active principles have four peak with retention time 3.37, 3.49, 4.0 and 5.69 in methanol as solvent at 254nm.
12. A process as claimed in claim 1 wherein the extraction process is carried out for a period ranging from 1 – 120 hrs, preferably between 12 – 16 hrs.
13. Pharmaceutical composition useful in the treatment of asthma, said composition comprising an effective amount of extract obtained from the plant *Murraya koenigii* together with, or optionally associated with a pharmaceutically acceptable additive.
14. A composition as claimed in claim 13 wherein the additives comprise powder or extracts of plants selected from *M. paniculate Linn*, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A.vasica Nees*, *E hirta*, and *M. koinegii*.
15. A composition as claimed in claim 13 wherein the additives are present in the range of 80-100 mg of *M. paniculate Linn*, 40-60 mg of *H. abelmoschus*, 38-62 mg of *T. ammi*, 7-13 mg of *S. aromaticum*, 85 - 115 mg of *A.vasica Nees* and 90-110 mg of *E hirta*.
16. A composition as claimed in claim 13 comprising :

<i>M. paniculata Linn. Syn. M exotica</i> (KAMINI)	90mg
<i>H. abelmoschus</i> (JOWAN)	50mg
<i>T. ammi</i> (LAVANGA)	50mg
<i>S. aromaticum</i> (BASAK)	10mg
<i>A.vasica Nees</i> (PUSITOA)	100mg
<i>E.hirta</i>	100mg
<i>M. koinegii</i> (Suravi Neem)	100mg

17. A composition as claimed in claim 13 wherein the extract of the plant *M. koinegii* is present in the range of 87-105 mg per dose.
18. A composition as claimed in claim 13 wherein the additives are preferably present in an amount 90 mg of *M. paniculate* Linn, 50 mg of *H. abelmoschus*, 50 mg of *T. ammi*, 10 mg of *S. aromaticum*, 100 mg of *A.vasica* Nees, 100 mg of *E hirta*, and 100 mg of *M. koinegii*. per dose.
19. A composition as claimed in claim 13 wherein the extract of the plant *M. koinegii* comprises active principles which are dark colored solids, soluble in dimethylsulfoxide.
20. A composition as claimed in claim 13 wherein the active principles have R_f values 0.73, 0.60, 034 and 0.14 in chloroform and methanol in the ratio 19:1 and R_f values 0.60, 0.38, 024 and 0.15 in the chloroform.
21. A composition as claimed in claim 13 having four peak with retention time 3.37, 3.49, 4.0 and 5.69 in methanol as solvent at 254nm.
22. A composition as claimed in claim 13 wherein the active principles obtained from the plant *M. koinegii* exhibit antioxidant property i.e. O_2 inhibition
23. A method for the treatment of asthma, said method comprising the steps of administering an effective amount of the composition as claimed in claim 13 to a subject in need thereof,
24. A method as claimed in claim 23 wherein the lyophilized extract obtained from *Murraya Koenigii* is administered along with other conventional additives for the treatment of asthma.
25. A method as claimed in claim 23 wherein the mode of administration is oral for the treatment of mild or acute asthma.
26. A method as claimed in claim 23 wherein the dosage level of the composition is in between 325-600 mg twice daily for the period ranging from 3 to 30 days.
27. A method as claimed in claim 23 wherein the dosage level is in between 325-600 mg twice daily for the period ranging from 3 to 15 days for mild asthmatic condition, and 15 – 30 days for acute asthmatic condition.
28. A method as claimed in claim 23, wherein the additives are selected from *M. paniculate* Linn, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A.vasica* Nees, *E hirta*, and *M. koinegii*.

29. A method as claimed in claim 23, where in the additives are present in a range of 80-100 mg of *M. paniculate* Linn, 40-60 mg of *H. abelmoschus*, 38-62 mg of *T. ammi*, 7-13 mg of *S. aromaticum*, 85-115 mg of *A.vasica* Nees, 90-110 mg of *E hirta*, and 87-105 mg of *M. koinegii*. per dose.
30. A method as claimed in claim 23, wherein the additives are preferably present in an amount 90 mg of *M. paniculate* Linn, 50 mg of *H. abelmoschus*, 50 mg of *T. ammi*, 10 mg of *S. aromaticum*, 100 mg of *A.vasica* Nees, 100 mg of *E hirta*, and 100 mg of *M. koinegii*. per dose.
31. A method as claimed in claim 23, wherein the additives *M. paniculate* Linn, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A.vasica* Nees, *E hirta*, and *M. koinegii* are administered to include properties such as antidiaphorol, antiseptic, carminative, stimulation, anti-cough, anti- bronchitis and nourishment.
32. A method as claimed in claim 23, wherein the additives are obtained from :
M. paniculate Linn (bark or root), *H. abelmoschus* from dried flower buds, *T. ammi* from leaves, *S. aromaticum* from whole plant *A.vasica* Nees from root, *E hirta* from bark, and *M. koinegii* from leaves.
33. An anti-oxidant composition for human beings and animals, said composition comprising a effective amount of extract obtained from the plant *Murraya Koenigii* together with or optionally, associated with pharmaceutically acceptable additives.
34. A composition as claimed in claim 33 wherein additives comprise powder or extracts of plants selected from *M. paniculate* Linn, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A.vasica* Nees, *E hirta*, and *M. koinegii*.
35. A composition as claimed in claim 33, wherein the additives are present in a range of 80-100 mg of *M. paniculate* Linn, 40-60 mg of *H. abelmoschus*, 38-62 mg of *T.ammi*, 7-13 mg of *S. aromaticum*, 85-115 mg of *A.vasica* Nees, 90-110 mg of *E hirta*, and 87-105 mg of *M. koinegii*. per dose.
36. A composition as claimed in claim 33, where in the additives are preferably present in an amount 90 mg of *M. paniculate* Linn, 50 mg of *H. abelmoschus*, 50 mg of *T. ammi*, 10 mg of *S. aromaticum*, 100 mg of *A.vasica* Nees, 100 mg of *E hirta*, and 100 mg of *M. koinegii*. per dose.

37. A composition as claimed in claim 33, wherein the additives *M. paniculate* Linn, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A. vasica* Nees, *E hirta*, and *M. koinegii* are added to provide properties namely, antidiaphoretic, antiseptic, carminative, stimulation, anti-cough, anti- bronchitis and nourishment, respectively.
38. A composition as claimed in claim 33, wherein the additives are selected from *M. paniculate* Linn, *H. abelmoschus*, *T. ammi*, *S. aromaticum*, *A. vasica* Nees, *E hirta*, and *M. koinegii* in the form of bark or root; seed; fruit; dried flower buds; leaves; whole plant; and root, bark, leaves, respectively.
39. Use of the extract obtained from the plant *Murraya koinegii* for the treatment of asthma.
40. An anti-asthma agent obtained from the plant *Murraya koinegii*.

ABSTRACT

The invention relates to a novel composition containing an extract obtained from the plant *Murraya koenigii* and useful for the treatment or providing relief from acute asthma, and a process for the preparation of a lyophilized extract containing active principles of the plant *Murraya koenigii*, and a method for the treatment of asthma.

002007-2009-060